

(19)

Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

**EP 0 997 199 A1**

(12)

**EUROPEAN PATENT APPLICATION**

(43) Date of publication:  
03.05.2000 Bulletin 2000/18

(51) Int. Cl.<sup>7</sup>: **B03D 1/02**, C07D 499/18,  
C07D 501/12, C12P 35/04,  
C12P 37/04

(21) Application number: **98203633.7**

(22) Date of filing: **26.10.1998**

(84) Designated Contracting States:  
**AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU**  
**MC NL PT SE**  
Designated Extension States:  
**AL LT LV MK RO SI**

(71) Applicant: **DSM N.V.**  
**6411 TE Heerlen (NL)**

(72) Inventors:  
• **De Vroom, Erik**  
**2313 JM Leiden (NL)**

• **Kers, Ernst Edmund**  
**3031 PM Rotterdam (NL)**  
• **Heijnen, Joseph Johannes**  
**5121 NR Rijen (NL)**

(74) Representative:  
**Visser-Luirink, Gesina, Dr. et al**  
**DSM Patents & Trademarks**  
**Office Delft**  
**P.O. Box 1**  
**2600 MA Delft (NL)**

(54) **Method for separation of solid compounds in suspension**

(57) A new method for separation of a partial suspension of a mixture of solid compounds into two or more single solid compounds and a mother liquor by the application of flotation has been described.

**EP 0 997 199 A1**

**Description****Field of the invention**

- 5 [0001] The present invention relates to a new method for the separation of one or more single solid compounds from a partial suspension of solid compounds by means of flotation.

**Background of the invention**

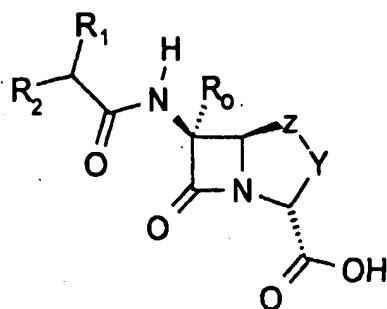
- 10 [0002] The most commonly used methods for the separation of organic end products from a mixture of solid compounds like starting materials and intermediates in a reaction mixture are either fractional crystallization, extraction or chromatography. However, these methods quite often lead to loss of valuable materials and overall higher costs of production thereof.
- 15 [0003] One of the less known techniques for selective separation of solids compounds is by means of flotation. The flotation technique is usually applied for metal ores concentration and in waste water treatment. The German patent document DE 4105384 mentions the selective flotation of phosphorus minerals from ores deposits by the addition of N-acylated protein, peptide or penicillin hydrolysate as the flotation reagent.
- [0004] Another German patent document DE 2621349 describes the separation technique of flotation by adding a flotation reagent and/or a gaseous medium to the mixture such as sludge, produced in municipal sewage or industrial waste treatment plants.
- 20 [0005] The object of the present invention is to provide a simple and widely applicable method, enabling organic compounds to be isolated from a mixture of solid compounds in pure form and without large loss of valuable materials.
- [0006] Surprisingly, it has been found that this can be achieved by subjecting a suspension or a partial suspension of the worked-up end product in a reaction mixture to flotation.
- 25 [0007] The method according to the present invention can suitably be applied for the separation of solid compounds used for medical purposes into two or more single solid compounds and a mother liquor such as for the production of  $\beta$ -lactam antibiotics.

**Description of the Figure**

- 30 [0008] A vertical column is fitted at the bottom with a Drainage outlet for pumping out the suspension of the products and at the top a Draining outlet for removing the foam generated during the flotation process. A partial suspension of solid compounds to be separated is inserted into the column via the Feed inlet. The gaseous phase is introduced into the column near to the bottom of the column via the Carrier Gas inlet and the washing liquid is passed through the
- 35 Washing Fluid inlet.

**Description of the invention**

- 40 [0009] The present invention describes a method for separation of a partial suspension of solid compounds into two or more single solid compounds and a mother liquor by the application of flotation on the suspension of a mixture of compounds in water and/or one or more organic solvents. The partial suspension of solid compounds refers to a suspension in water and/or organic solvent of solid compounds in which a part of the same is in a soluble state and the rest in a solid state. The solid compounds to be separated can be produced by a chemical process and/or an enzymatic process. The process of flotation can be applied in a separation vessel, separation funnel or column, preferably in a column.
- 45 [0010] The flotation is achieved by one or more of the measures selected from shaking, stirring and introducing a gaseous phase into the suspension. Preferably the gaseous phase is compressed air or nitrogen. The gaseous phase can be introduced into the lower 40% part of the column, preferably at the bottom of the same. Preferably the mother liquor is recirculated into the suspension of the mixture of compounds.
- 50 [0011] For the sake of illustration, the process of flotation in a column is depicted in the figure enclosed.
- [0012] The compounds which can be separated from the mixture of compounds suspended in one or more solvents are organic compounds, preferably a  $\beta$ -lactam compound with the general formula (I): with



(II)

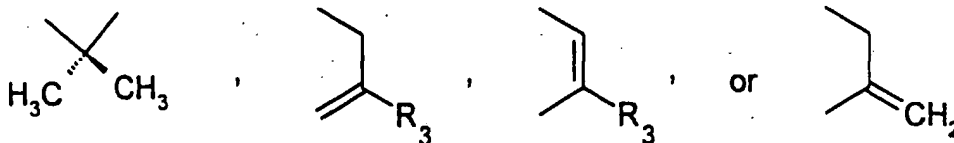
$R_0$  is hydrogen or  $C_{1-3}$  alkoxy;

$R_1$  is hydrogen, hydroxy, amine, halogen or lower alkyl;

$R_2$  is an optionally substituted phenyl or phenoxy or 5- or 6-membered heterocyclic ring;

$Z$  is oxygen, sulphur or an oxidized form of sulphur or  $CH_2$  or optionally substituted  $CH_2$ ; and

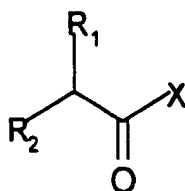
$Y$  is



wherein

$R_3$  is hydrogen, hydroxy, halogen,  $C_{1-3}$ alkoxy, optionally substituted, optionally containing one or more heteroatoms, saturated or unsaturated, branched or straight  $C_{1-5}$ alkyl, preferably methyl, optionally substituted, optionally containing one or more heteroatoms,  $C_{5-8}$ cycloalkyl, optionally substituted aryl or heteroaryl, or optionally substituted benzyl.

**[0013]** For the preparation of  $\beta$ -lactam antibiotics, a  $\beta$ -lactam nucleus such as 6-aminopenicillanic acid (6-APA), 7-aminocephalosporanic acid (7-ACA), 7-amino-3-chloro-3-(desacetoxyethyl)cephalosporanic acid (7-ACCA), 7-amino-3'-desacetylcephalosporanic acid (7-ADAC), or 7-amino-3'-desacetoxycephalosporanic acid (7-ADCA) is acylated with an amino acid such as D-(-)-phenylglycine (PG), D-(-)-4-hydroxyphenylglycine (HPG) or (-)-4-hydroxyphenylglycine methyl ester (HPGM). The acylation can be performed by means of chemical activation such as described in European patent application EP 011513, United States patent US 4,248,780, German patent applications DE 1302847, DE 2020133, DE 2065879 and British Patents GB 1327270 and GB 1347979. The acylation can also be done by using enzymatic catalysis such as described in European patent application EP 730036 and International patent application WO 96/23897. An activated side chain of formula (II) can be used for the enzymatic acylation of a  $\beta$ -lactam nucleus:



**10.**

15

20

25

35

40

45

56

## Definitions

5

**Example 1**

**Separation of ampicillin and phenyl glycine from a partial suspension resulting from the enzymatic acylation reaction in which the suspension from the bottom exit of the column resulting during the flotation is recirculated back into the buffer vessel.**

STEP A: Preparation of a solution of  $\text{PGA}(\text{H}_2\text{SO}_4)_{1/2}$ 

[0020] 301.6 g PGA (2.00 mol) is suspended in 650 g water at 5°C. 102.1 g 96%  $\text{H}_2\text{SO}_4$  (1.00 mol) is added under stirring in one hour. During this process, the temperature is maintained below 25°C.

STEP B: Enzymatic synthesis of Ampicillin

[0021] An enzymatic reactor (1.5 l vessel, diameter 0.11 m, equipped with a 175  $\mu\text{m}$  mesh sieve-bottom) is charged with 300 g Assemblas<sup>®</sup> (obtained by separating the immobilized enzyme from an enzyme slurry, washing the beads with water, and weighing the beads without drying). In a second vessel (1.2 l) 131.6 g 6-APA (0.600 mol), 30.2 g PGA (0.200 mol) and 400 ml water is stirred for 15 min at 10°C and then transferred to the enzyme reactor using 100 ml water as transferring fluid. At 10°C, the enzyme reactor is supplied with 423.7 g  $\text{PGA}(\text{H}_2\text{SO}_4)_{1/2}$ -solution from STEP A (0.800 mol) during 233 min using a constant speed in such a way that the pH is maintained at 6.3. After 295 min, the pH is maintained at 6.3 by means of titrating with a 12 M solution of  $\text{H}_2\text{SO}_4$ . After 570 min the pH is lowered to 5.0 using a 12 M solution of  $\text{H}_2\text{SO}_4$ . The enzyme reactor now contains 575 mmol Ampicillin, 15 mmol 6-APA, 50 mmol PGA and 365 mmol PG.

STEP C: Removal of the Ampicillin/PG slurry from the enzyme reactor

[0022] The slurry of Ampicillin and PG as obtained in STEP C is removed from the enzyme reactor using filtration in combination with stirring at 500 rpm. The reactor is rinsed with 10 x 250 ml water (10° C). The resulting white slurry contains > 99.8% of the Ampicillin produced and > 99.5% of the PG produced. The Assemblase<sup>®</sup> is retained in the enzyme reactor for > 99.5%.

STEP D: Separation of Ampicillin and PG using flotation in a column

[0023] In this example a glass column is used with a length of 230 cm and an internal diameter of 2 cm.

[0024] The column (length = 230 cm and internal diameter = 2 cm) is fed (flow 1.5 l/h) from a buffer vessel at a height of 96 cm with a suspension of STEP C. Through the column air is blown with a flow of 2 l/h. The suspension from the bottom exit is passed back into the buffer vessel. The foam that is formed on the top of the column is removed from the system. After 18 hours the composition of the solid material in the buffer vessel has changed from 65% Ampicillin and 16% PG to 86% Ampicillin and 4% PG. At that point, the material leaving the bottom exit contains 86% Ampicillin and 0% PG.

**Example 2**

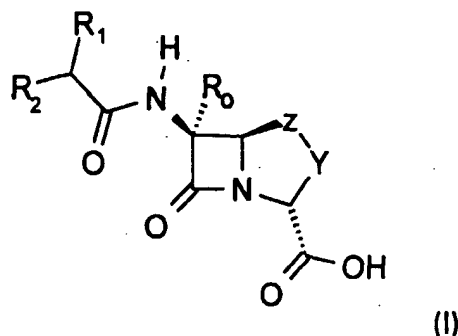
**Separation of ampicillin and phenyl glycine from a partial suspension resulting from the enzymatic acylation reaction in which the suspension from the bottom exit and the foam at the top of the column resulting during the flotation is recirculated back into the buffer vessel.**

Separation of Ampicillin and PG using flotation in a column

[0025] The same column is used as in Example 1. The column is fed from a buffer vessel at a height of 96 cm with a suspension produced in a similar method as described in the Example 1. Through the column air is blown with a flow of 2 l/h. The suspension which is drained from the column at the bottom (flow 300 ml/h) is continuously filtrated and the mother liquor is returned to the top of the column. The foam that is formed on the top of the column is continuously passed back to the buffer vessel. The solid material in the suspension in the buffer vessel had at the start a composition of 69% Ampicillin and 18% PG. The crystal cake isolated from the bottom of the column contains 85% Ampicillin and 0% PG. The foam formed in the top contains 53% Ampicillin and 40% PG.

## Claims

1. A method for separation of a suspension of solid compounds into two or more single solid compounds and a mother liquor, characterised by the application of flotation on the suspension of a mixture of compounds in water and/or one or more organic solvents.
2. A method according to claim 1, wherein the flotation is applied in a separation vessel, separation funnel or column.
3. A method according to claim 2, wherein preferably the flotation is applied in a column.
4. A method according to any one of the claims 1-3, wherein the flotation is achieved by one or more of the measures selected from shaking, stirring and introducing a gaseous phase into the suspension.
5. A method according to claim 4, wherein the gaseous phase is introduced into the lower 40% part of the column, preferably at the bottom of the same.
6. A method according to claim 5, wherein the gaseous phase is air or nitrogen.
7. A method according to any one of the claims 1-6, wherein the mother liquor is recirculated into the suspension of the mixture of compounds.
8. A method according to claims 1-7, wherein one of the compounds is an organic compound, preferably a  $\beta$ -lactam compound of general formula (I):



with

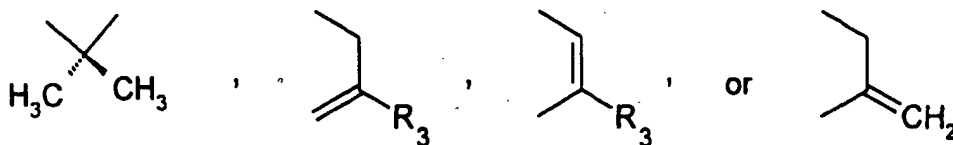
$R_0$  is hydrogen or  $C_{1-3}$  alkoxy;

$R_1$  is hydrogen, hydroxy, amine, halogen or lower alkyl;

$R_2$  is an optionally substituted phenyl or phenoxy or 5- or 6-membered heterocyclic ring;

Z is oxygen, sulphur or an oxidized form of sulphur or  $CH_2$  or optionally substituted  $CH_2$ ; and

Y is

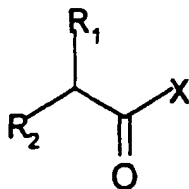


wherein

$R_3$  is hydrogen, hydroxy, halogen,  $C_{1-3}$  alkoxy, optionally substituted, optionally containing one or more hetero-

oatoms, saturated or unsaturated, branched or straight C<sub>1-5</sub>alkyl, preferably methyl, optionally substituted, optionally containing one or more heteroatoms, C<sub>5-8</sub>cycloalkyl, optionally substituted aryl or heteroaryl, or optionally substituted benzyl.

- 5 9. A method according to claim 8, wherein the mixture of compounds containing the  $\beta$ -lactam compound is prepared by an enzymatic condensation reaction of a  $\beta$ -lactam nucleus with an activated side chain of formula (II): with



(II)

20 R<sub>1</sub>, R<sub>2</sub> is defined as in claim 8, X is NHR<sub>3</sub>, wherein R<sub>3</sub> is hydrogen or lower alkyl or X is alkoxy, preferably lower alkoxy and more preferably methoxy.

- 25 10. A method by using enzymatic condensation according to claim 9, wherein the mixture contains,

10-1500 mM, preferably 50-1000 mM of a  $\beta$ -lactam antibiotic,  
0-1500 mM, preferably 50-1000 mM of hydrolyzed side chain,  
0-1000 mM, preferably 0-200 mM of  $\beta$ -lactam nucleus, and  
0-1000 mM, preferably 0-400 mM of activated side chain.

- 30 11. A method according to claims 6-8, wherein the  $\beta$ -lactam antibiotic is chosen from the group consisting of ampicillin, amoxicillin, epicillin, cephalixin, cefaclor, cefadroxil, cefradrin, cefroxadine, cefprozil and loracarbef.

- 35 12. Use of the flotation method to improve a separation process of a compound from a suspension of solid compounds.

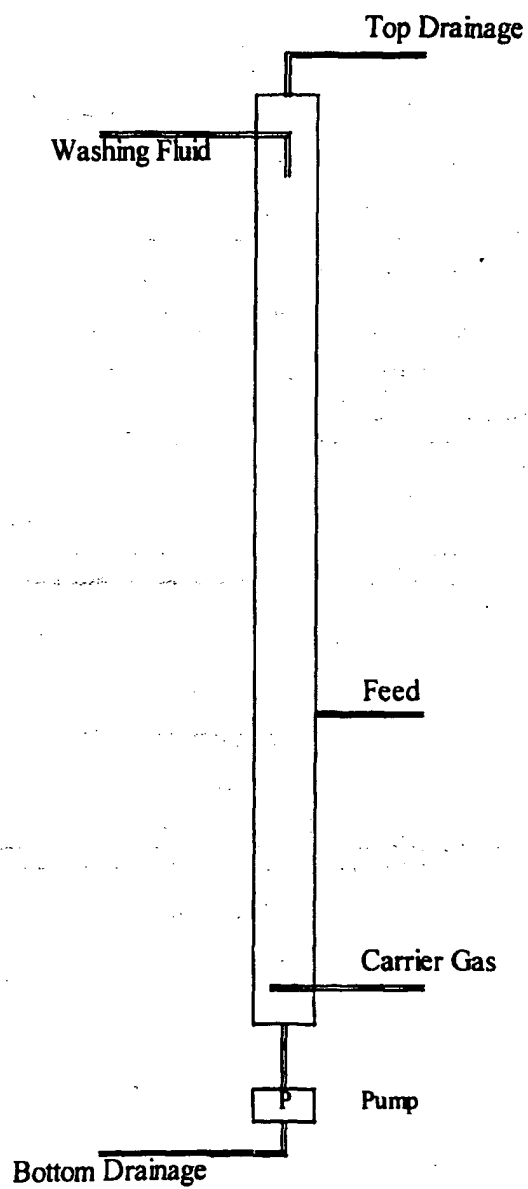
40

45

50

55

Figure





European Patent  
Office

# EUROPEAN SEARCH REPORT

Application Number  
EP 98 20 3633

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
D,A	DE 41 05 384 A (HOECHST AG) 29 August 1991 * claims *	1-6,12	B03D1/02 C07D499/18 C07D501/12 C12P35/04 C12P37/04
A	DE 22 59 020 A (HARTLEY SIMON LTD) 14 June 1973 * claims *	1-6,12	
A	CHEMICAL ABSTRACTS, vol. 97, no. 2, 12 July 1982 Columbus, Ohio, US; abstract no. 8483, KABRT J.: "Separation of solids from suspensions " XP002097011 * abstract * & CS 193 314 A	1,12	
A	WO 92 12782 A (NOVONORDISK AS) 6 August 1992 *page 5; page 6, lines 30-33; examples 2,3 and claims*	7-11	
A	GB 994 402 A (BAYER AG.) 10 June 1965 * the whole document *	7-11	<b>TECHNICAL FIELDS SEARCHED (Int.Cl.6)</b>  B03D C07D C12P
The present search report has been drawn up for all claims			
Place of search <b>THE HAGUE</b>		Date of completion of the search <b>18 March 1999</b>	Examiner <b>Chouly, J</b>
<b>CATEGORY OF CITED DOCUMENTS</b> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document  T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons  & : member of the same patent family, corresponding document			

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 98 20 3633

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

18-03-1999

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
DE 4105384 A	29-08-1991	NONE	
DE 2259020 A	14-06-1973	GB 1408878 A	08-10-1975
		AT 324970 B	25-09-1975
		AU 471695 B	29-04-1976
		AU 4943972 A	30-05-1974
		BE 792423 A	30-03-1973
		CA 965731 A	08-04-1975
		FR 2162649 A	20-07-1973
		HK 47976 A	30-07-1976
		JP 837303 C	15-12-1976
		JP 48066003 A	11-09-1973
		JP 51012281 B	17-04-1976
		NL 7216328 A	13-06-1973
		NL 7701178 A	29-07-1977
		US 3817865 A	18-06-1974
		ZA 7208507 A	31-07-1974
WO 9212782 A	06-08-1992	AU 1235992 A	27-08-1992
		BG 97981 A	25-04-1994
		CA 2101256 A	26-07-1992
		CZ 9301485 A	19-01-1994
		EP 0569462 A	18-11-1993
		HU 67012 A	30-01-1995
		JP 6504947 T	09-06-1994
		MX 9200313 A	30-10-1992
		SK 78593 A	12-01-1994
GB 994402 A		NONE	

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ **BLACK BORDERS**
- ☐ **IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- ☐ **FADED TEXT OR DRAWING**
- ☐ **BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- ☐ **SKEWED/SLANTED IMAGES**
- ☐ **COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- ☐ **GRAY SCALE DOCUMENTS**
- ☐ **LINES OR MARKS ON ORIGINAL DOCUMENT**
- ☐ **REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- ☐ **OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**